ANOTHER CASE OF MULTIPLE ALLELOMORPHS IN DROSOPHILA.

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It has been shown[1] that the factor for white eye color and the factor for eosin eye color are allelomorphs. Each arose as a mutation, but unlike other original and independent mutations they do not give, when crossed, the wild type (red eye) by recombination. Each factor has the same normal allelomorph in the wild fly, and each factor gives the same linkage ratio as the other one with any sex-linked factor. The factor for eosin, the factor for white and their normal allelomorph form a system of triple allelomorphs. Only two of these factors can exist at any one time in the same female, and, since they are sex-linked, only one at a time in a male. In consequence, a red-eyed female may be heterozygous for white or for eosin, but never for white and for eosin.

Another similar case has also appeared. In certain crosses, involving yellow body color, a yellowish male appeared that was characterized by a light spot on the upper surface of the posterior end of the abdomen (Figs. 3 and 4). When the yellow stock was examined another male of the same kind was found there. It is evident that in the first case the new type must have come from the original yellow stock. The mutation probably occurred in the stock one or more generations before it was discovered.

It was natural to assume that such a peculiar spot could be transferred to gray or to black flies. The original male and later many of his offspring that bore the same character were mated to gray and to black flies. The first generation was always normal gray. In the second generation the yellows and the yellow blacks ("browns") were spotted, but never any of the gray or the black flies. Thinking that the spotting was closely linked to the yellow factor and might cross over if enough flies were bred I continued the experiment for four or five months.

[1] Morgan and Bridges, *Journ. Exp. Zool.*, XV., 1913.

While no record of the number of flies that were examined was kept, I think more than 30,000 gray and black flies must have been looked over. In no case was the spot present.

The alternatives then are absolute (or possibly nearly absolute) linkage, or a case of multiple allelomorphism. In practice these alternatives would give the same numerical results. The theoretical differences between the idea of absolute linkage and multiple allelomorphism will be discussed later.

When a spot male was mated to wild females, all the offspring were normal gray without spot. When the F_1 offspring were inbred the following classes were produced.

	Gray ♀.	Gray ♂.	Spot ♂.
a.	285	132	94
b.	305	127	113
Total...........	590	259	207

There is one class of females and two classes of males. This is the expectation if spot is due to a sex-linked factor. It is the same kind of result that obtains when a yellow male is crossed to a wild female. When an F_1 female from the last experiment was back crossed to a spot male the following results were obtained:

	Gray ♀.	Spot ♀.	Gray ♂.	Spot ♂.
a.	100	88	91	84
b.	25	16	15	20
c.	86	87	70	48
Total.....	211	191	176	152

Equality in all four classes is expected and this is practically realized. The spot type seems somewhat less viable than the wild type. The male counts run behind the female, which is a usual result. Here the spot also occurs in the female (Figs. 7 and 8).

A spot female was mated to a yellow male. The 63 daughters were yellow without spot which shows that spot is completely recessive to yellow. The 58 sons were spot, receiving their sex chromosome from the mother. When a pair of these F_1 flies were bred, the following F_2 classes were produced.

Yellow ♀.	Spot ♀.	Yellow ♂.	Spot ♂.
44	45	41	45

This is the expected result for this combination.

The body-color black belongs to our second group of linked factors. A spot female was mated to a black male. The daughters were gray without trace of spot, the sons were spot. Female producing sperms bring in the normal allelomorph for spot (Y), which, with the normal allelomorph for black (B), gives gray. The sons are spot because the male receives his sex chromosome from his mother. The formulas for the F_1 and F_2 generations are the following:

Spot ♀, $y_sB—y_sB$.
Black ♂, Yb ——b.

F_1 { Gray ♀, $y_sB\ Yb$.
Spot ♂, $y_sB—b$. }

$y_sB—Yb—\ y_s\,b—\ YB$, Eggs of } F_1.
$y_sB—y_sb$——B——b, Sperm of } F_1.

y_sBy_sB,	spot.	$y_sB—B$,	spot.
Yby_sB,	gray.	Yb —B,	gray.
y_sby_sB,	spot.	y_sb —B,	spot.
YBy_sB,	gray.	$YB—B$,	gray.
y_sBy_sb,	spot.	$y_sB—b$,	spot.
Yby_sB,	black.	Yb —b,	black.
y_sby_sb,	spot black.	y_sb —b,	spot black.
YBy_sb,	gray.	$YB—b$,	gray.

The F_2 expectation is 3 gray, 3 spot, 1 black, 1 spot black, and is the same for the male and the female classes which are counted together in the following F_2 results for this cross.

F_2 Females.		F_2 Males.	
Gray.	Spot.	Black.	Spot Black.
133	90	47	39

The double recessive is spot black, which corresponds in color to yellow black or "brown" of earlier papers. The light spot shows here even more strikingly, because of contrast, than in the simple "spot" itself.

Pure stock of the double recessive spot black being on hand, a male was crossed to a yellow female. The F_1 were yellow flies, 65 males and 20 females without spot, showing again the dominance of yellow to spot in the "compound" y-y_s. These F_1 flies inbred gave:

Yellow ♀.	Yellow Black ♀.	Yellow ♂.	Yellow Black ♂.	Spot Black ♂.	Spot ♂.
126	37	33	26	11	26

A male of the spot black stock was also bred to a black female. The offspring were black males and females, which inbred in four lots gave the following results:

	Black ♀.	Black ♂.	Spot Black ♂.
1.	313	165	109
2.	291	140	75
3.	396	211	85
4.	494	255	144
5.	363	178	115
Totals...........	1,857	949	528

In these mass cultures, although an attempt was made to have the food conditions favorable, yet, owing to crowding, the spot black males ran far behind the black males.

There appeared in the above F_2 a yellow black fly with no trace of spot. Since in some cultures (but not here) the spot may be faint and difficult to detect with certainty, it seemed possible that the fly was such a *somatic* variant. He was tested by breeding to a black female. The offspring were black (very few in number). These inbred gave:

	Black ♀.	Black ♂.	Yellow Black ♂.
1	55	19	32
2	316	172	151
3	59	42	29
Total..........	430	233	212

Had the male in question been only a somatic condition of yellow (suppressed spot) we should have expected some spot males in the F_2 generation. Since none at all appeared there can be no doubt that the grandparent was in reality a *yellow* black individual both somatically and gametically. The most prob-

able explanation is that a mutation backwards from spot to yellow took place (with no change in the black). Another interpretation is that contamination had occurred. This seems highly improbable since at the time there was no stock of yellow black in the laboratory, and only a few experiments under way in which such individuals were being produced. Nevertheless, so long as there were no other characters than these in the experiment by means of which the offspring could be further identified, I think that one cannot be too careful in interpreting such rare occurrences as mutation backwards. A third possibility is that a cross over occurred, and if so, spot is closely linked to yellow, and crossed over only in this one case out of the many thousands of cases.

In order to see whether the spot would show in the female of the "compound" yellow-spot, when the compound was also black, a spot black female was bred to a yellow black male. All the daughters were yellow black without trace of spot. The sons were spot black. These mated together gave the following F_2 generation.

	Yellow Black ♀.	Spot Black ♀.	Yellow Black ♂.	Spot Black ♂.
1.	15	29	18	25
2.	45	47	42	25
3.	60	62	61	51
Totals....	120	138	121	101

Other Crosses with Spot.

We have another stock of black-colored flies called ebony which belongs to our third group of factors. When spot female was crossed to ebony male the daughters were gray and the sons were spot. These inbred gave in F_2 the following results.

Gray.	Spot.	Ebony.	Spot Ebony.
91	102	29	24
106	73	45	28

The analysis is the same as in the case of spot by black. The double recessive is spot ebony which has a brownish color differing in tint from the brown of spot black.

There is still another black-colored mutant called sable. It is

sex-linked, and belongs, therefore, in our first (or sex-linked) group of factors. When a spot female was bred to sable male the daughters were gray and the sons spot. Inbred these gave the following results.

Gray ♀.	Spot ♀.	Spot ♂.	Sable ♂.	Gray ♂.	Spot Sable ♂.
35	30	17	20	24	8

The analysis is the same as when the cross is made with yellow, except that gray is formed by recombination here. The linkage of spot and sable modifies the ratios somewhat.

In the cross with ebony and in that with sable there appeared in the F_1 generation a few exceptional cases that are due to non-disjunction. The actual results are

	Spot ♀.	Gray ♀.	Spot ♂.	Gray ♂.
F_1	0	112	104	3
	0.	66	52	4

	Spot ♀.	Gray ♀.	Spot ♂.	Sable ♂.
F_1	2	62	64	2

These cases are like those described by Bridges and call for no further comment here. They are explicable on the assumption that the two sex chromosomes of certain spot females occasionally stick together, and either pass out of the egg or else both remain in it. In the first work done more than a year ago with spot, some unexplained results were obtained but not then interpreted. It now appears that in part at least they may have been due to non-disjunction.

The Origin of Multiple Allelomorphs.

It is noteworthy that spot appeared in yellow stock. It may seem that there is some causal connection here since spot is a mutation in the same locus as yellow. Likewise eosin appeared in white stock, and is another case of multiple allelomorphism. Plausible as is the assumption it can not be proven, but the following considerations are not without interest. If the mutation appeared first in the yellow stock, in a chromosome that went into a male, the spot would be apparent on examination. If the chromosome went into a female the mutation would not appear for another generation and then in a male. If on the other hand the mutation had appeared in a chromosome in the gray stock

it might appear in the next generation if it went into a male, just as yellow itself might have appeared in the same way. If the chromosome in question had passed into a female no evidence of the mutation would be seen, but it would appear in the second generation males. The two cases are alike. Since, however, the mutation has never appeared in the gray stock, although a great many more gray flies have been seen than yellow flies, it might be argued that a mutation in a locus is more likely to be followed by another in the same locus than if no mutation had taken place there. But mutations are such rare phenomena that this argument does not carry much weight, and judgment must be suspended until further mutations have shown themselves.

Other Characteristics of Spot.

In general we are apt to seize upon the most evident characteristic of a mutation, give it a name, and neglect to mention, or overlook, other effects that may be associated with it. In the case of the white eye mutation for instance the eye attracts our attention, yet if red-eyed and white-eyed flies be put into alcohol the yellow, especially the yellow bands, become markedly different in the two flies. The white-eyed flies show much whiter bands. Evidently the mutation has affected other pigments than those contained in the eye.

In another mutation called club wing (as yet undescribed) the wing pads of many flies fail to expand, although others do expand even in pure stock. It was later found that a small bristle on each side of the thorax is absent in the club stock irrespective of whether the wing pads expand or not. This microscopic change is a constant feature of the mutation and a better character for that reason than the more obvious although inconstant one of the club-shaped wing.

Likewise in "spot." In the black spot fly there is a distinct light dot on the post thorax, and often a light band down the middle of the thorax (Fig. 4a). These were called dot and dash, but it was later noticed by Bridges that the dot and dash are present in flies only for a short time and disappear as the flies get older. All attempts to get stocks with permanent dot and dash

failed, because, as we now think, these are only juvenile characters. The dot is sometimes seen in young spot flies, but is weak and soon disappears. Dot and dash are, therefore, due to the same mutation that gave the spot, but are ephemeral and are especially seen in the double recessive condition—black spot. The different degrees of viability shown by the different mutations may also be looked upon as effects associated with the mutation—effects of the highest importance in determining the chances of survival of the mutants, but of small value as indicators of the mutation itself.

Linkage of Spot and White.

If, as the evidence shows, spot is an allelomorph of yellow, and both have a common normal allelomorph, it was not considered worth while to carry out any extensive experiments in linkage, since the data for yellow would apply to spot. Incidentally, however, a few experiments were made with white-eyed flies, and may be given here.

A few yellow white males were bred to spot red-eyed females. The F_1 flies were yellow red females and spot red males. These inbred gave:

	Yellow Red ♀.	Spot Red ♀.	Yel. White ♂.	Spot Red ♂.	Yel. Red ♂.	Spot White ♂.
(1)	63	19	40	31	0	1
(2)	94	21	47	60	1	0

The linkage of spot and white shown by these figures is 1.11. That already determined for yellow white is 1.12. The coincidence is greater than might be expected with small numbers for loci so near together.

In another experiment a spot black, red-eyed male was mated to a yellow white-eyed female. The daughters were yellow red, the sons yellow white flies. These inbred gave:

Females.

Yellow Red.	Yellow Black Red.	Yellow White.	Yellow Black White.
45	11	41	9

Males.

Spot Red.	Spot-black Red.	Yellow White.	Yellow-black White.
39	13	33	12

The analysis follows:

Yellow white ♀, $ywBywB$.
Spot black ♂, y_sWbb.
ywB— ywB, Eggs of $\left.\right\}$ P_1.
y_sWb— —b, Sperm of

F₁ ♀, $ywBy_sWb$.
F₁ ♂, ywB—b,

Eggs yWb—y_sWB—yWB—ywB—y_sWb—y_swb—ywb—y_swB,
Sperm _____ ywB—ywb— ——B— ——b, __________.

F_2 Females.		F_2 Males.	
$yWbywB$,	yellow red.	yWb —B,	yellow red.
y_sWBywB,	yellow red.	y_sWB—B,	spot red.
$yWBywB$,	yellow red.	yWB —B,	yellow red.
$ywBywB$,	yellow white.	ywB —B,	yellow white.
y_sWbywB,	yellow red.	y_sWb —B,	spot red.
y_swbywB,	yellow white.	y_swb —B,	spot white.
$ywbywB$,	yellow white.	ywb —B,	yellow white.
y_swBywB,	yellow white.	y_swB —B,	spot white.
$yWbywb$,	yellow black red.	yWb —b,	yellow black red.
y_sWBywb,	yellow red.	y_sWB—b,	spot red.
$yWBywb$,	yellow red.	yWB —b,	yellow red.
$ywBywb$,	yellow white.	ywB —b,	yellow white.
y_sWbywb,	yellow black red.	y_sWb —b,	spot black red.
y_swbywb,	yellow black white.	y_swb —b,	spot black white.
$ywbywb$,	yellow black white.	ywb —b,	yellow black white.
y_swBywb,	yellow white.	y_swB —b,	spot white.

As shown by the preceding analysis, the expectation, without considering the strong linkage between spot and white, is as follows:

Females.	
Yellow red	6
Yellow white	6
Yellow-black red	2
Yellow-black white	2

Males.	
Yellow red	3
Yellow white	3
Yellow-black red	1
Yellow-black white	1
Spot red	3
Spot white	3
Spot-black red	1
Spot-black white	1

The four classes of females were realized in the results, but only four of the possible eight classes of males appeared. These four are the non-cross-over classes between spot and white; those absent are the crossovers. Considering the small numbers involved, the absence of these four classes of males is a probable event.

EXPLANATION OF PLATE I.

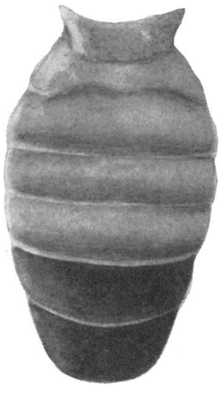
FIG. 1. Normal abdomen of male, dorsal view.

FIG. 2. Ditto, side view.

FIG. 3. Spot abdomen of male, dorsal view.

FIG. 4. Ditto, side view.

FIG. 4*a*. Thorax (dorsal view) of young spot black showing dot and dash.



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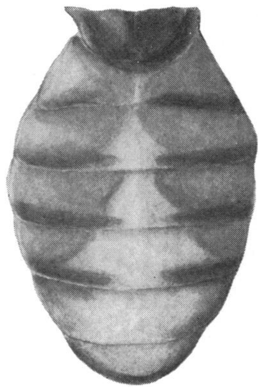

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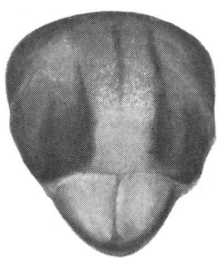

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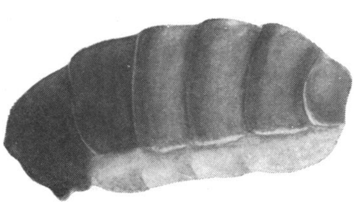

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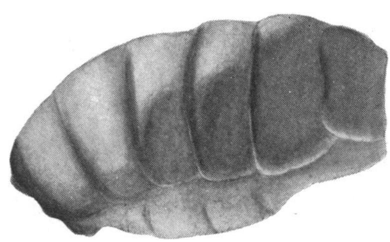

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EXPLANATION OF PLATE II.

FIG. 5. Normal abdomen of female, dorsal view.
FIG. 6. Ditto, side view.
FIG. 7. Spot abdomen, dorsal view.
FIG. 8, Ditto, side view.

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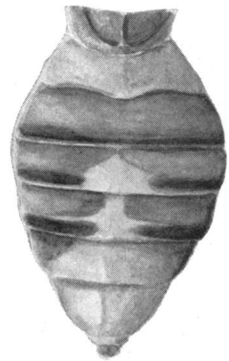

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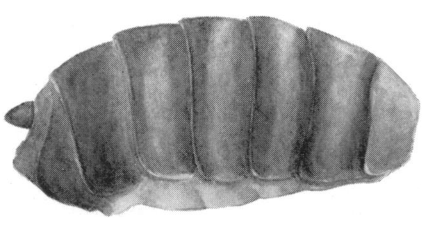

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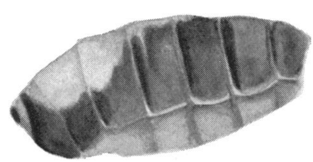

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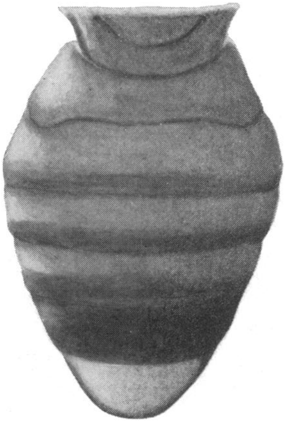
EXPLANATION OF PLATE III.

FIG. 9. Spot abdomen of male showing slight development of spot, dorsal view.

FIG. 10. Ditto, side view.

FIG. 11. Ditto, female, dorsal view.

FIG. 12. Ditto, side view.



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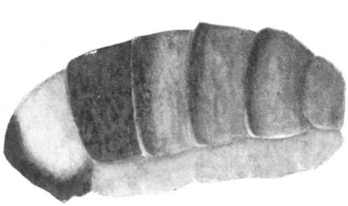

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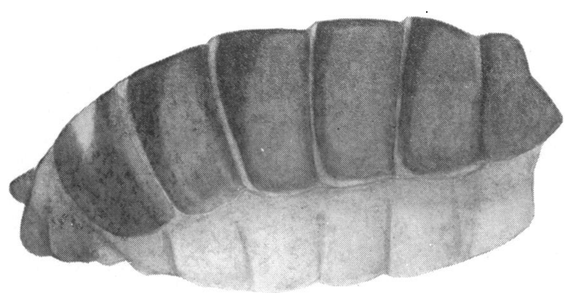

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